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Possible Causes of The Damage to Chromosomes in Aged Seeds

H. Özkan SİVRİTEPE*

SUMMARY

Different hypotheses have been reviewed, to update the knowledge on the cause of the damage to chromosomes as seeds age. These hypotheses (i.e., the presence of background irradiation, accumulation of automutagenic substances, lipid autoxidation and loss of DNA integrity) have been given in historical order. Although each hypothesis gives an idea on the damage to chromosomes in aged seeds, it is unfortunate that there is no direct evidence to show what exactly affects this phenomenon.

Key words: Damage to chromosomes, aged seeds.

ÖZET

Yaşlanmış Tohumlarda Kromozomların Zararlanmasının Muhtemel Nedenleri

Tohumlar yaşlanırken kromozomlarda meydana gelen zararlanmanın nedeni hakkındaki bilgilerin güncelleştirilmesi için farklı hipotezler incelenmiştir. Bu hipotezler (radyoaktif ışınların varlığı, otomutagenik maddelerin birikimi, yağlarda meydana gelen oksitlenme ve DNA bütünlüğünün kaybı) tarih sırasıyla verilmiştir. Her hipotez yaşlanmış tohumlarda kromozomların bozulması

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effective on flower formation when applied at proper time. Other than the removal of the irregularity in yield, an advantage of such chemical substances is the improvement in fruit quality. However, it is compulsory to be careful by health when using these substances.

The studies realted to decreasing the severity of alternation by use of chemical substances are quite recent in Turkey. The application of growth regulating chemical substances along with other cultural techniques in such studies, no doubt, is important in achievment of our goal in a short time. On the other hand, the investigations on chemical girdling nearby classical methods will be beneficial not only for olive but also for other fruit species that require girdling.

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hakkında bir fikir vermesine rağmen, bu olayı neyin etkilediğini göstermek için direkt delillerin bulunmayışı da bir talihsizliktir.

Anahtar sözcükler: Kromozomların zararlanması, yaşlanmış tohumlar.

INTRODUCTION

Although a number of hypotheses have been proposed to explain the cause of the damage to chromosomes as seeds age, what exactly affects this phenomenon is as yet unclear. These hypotheses include: the presence of background irradiation, accumulation of automutagenic substances, lipid autoxidation, to name a few. Previously, Roberts (1972), Abdul-Baki and Anderson (1972), Heydecker (1973), Bewley and Black (1985) and Priestley (1986) have reviewed these hypotheses. This review was carried out to update the knowledge on this phenomenon.

1. BACKGROUND IRRADIATION

The discovery of the mutagenicity of x-rays and other radioactive sources led to the hypothesis that a gradual accumulation of chromosome damage in seeds stored under normal conditions resulted from normal background radiation. However, Giles (1940) pointed out that the cause of spontaneous chromosome aberration in *Tradescantia* could not be explained by background radiation. Similarly, Gunthardt et al. (1953) concluded that the dosage of natural radiation, including cosmic radiation, received by seeds in storage was insufficient to account for the frequencies of cytogenetic changes observed in the aged seeds.

2. AUTOMUTAGENIC SUBSTANCES

Later, another hypothesis which attempted to explain the spontaneous mutability of aged seeds on a metabolic basis gained popularity. The idea that chromosome damage is a consequence of the accumulation of automutagenic substances resulting from normal metabolism of seeds during ageing received a lot of attention.

Two techniques were utilized to detect mutagenic substances. The first involved plant extracts in which fresh seeds were treated with extracts from aged seeds of the same or different species has been reviewed in detail by D'Amato and Hoffman-Ostenhof (1956). Automutagenic activity of seed extracts has been reported by several authors, e.g. Gisquet et al. (1951) in *Nicotiana tabacum,*

Keck and Hoffman-Ostenhof (1952) in *Nicotiana tabacum*, Keck and Hoffman-Ostenhof (1952) in *Phaseolus vulgaris*, Kato (1954) in *Allium fistulosum* and Jackson (1959) in *Allium cepa*. However, several authors found no effects of seed extracts on seeds of the same species, e.g. Marquardt (1949a, b) in *Oenothora* sp., Gori (1953) in *Reseda odora*, D'Amato (1953) in *Pisum sativum* and Abdalla and Roberts (1968) in *Hordeum distichon*, *Vicia faba* and *Pisum sativum*.

The second technique involved reciprocal transplants of embryos and endosperms between old and young seeds. Nuti Ronchi and Martini (1962) transplanted embryos from fresh wheat seeds onto endosperms from aged seeds with results suggesting the existence of chemical mutagens in the endosperms. However, Corsi and Avanzi (1969) found that the incidence of chromosomal aberrations in the embryos induced by ageing is not the result of senescence in the endosperm although low levels of chromosome damage can be induced by old endosperms. Recently, Floris and Anguillesi (1974) concluded that aged embryos and endosperms produced mutagenic substances capable of inducing nuclear damage in the radicle meristem. However, they also conluded that age-induced damage in the embryos was not the consequence of endosperm ageing. Therefore, the attempts of explaining the possible cause of chromosomal aberrations in aged seeds by the use of automutagenic substances were obscured.

3. LIPID AUTOXIDATION

Several attempts to explain the increase in chromosomal aberrations in aged seeds that focused on lipid autoxidation have engendered much speculation. However, it is still questionable whether such changes are necessarily linked to seed ageing. The lipid autoxidation hypothesis involves the following steps (Harrington 1973):

1. Unsaturated fats $\xrightarrow[\text{light and other irradiation}]{\text{metal ions}}$ free radicals

2. Free radicals + O_2 $\longrightarrow$ hydroperoxides

3. Hydroperoxides $\longrightarrow$ carbonyls

4a. Carbonyls and nucleic acids ⟶ inactivation of enzymes, membrane injury and histone denaturation

4b. Carbonyls and nucleic acids ⟶ chromosomal mutation

A variety of enzymatic and spontaneous oxidations generates the free superoxide radical (O_2), which is cytotoxic and which can react with H_2O_2 to produce singlet oxygen and the hydroxyl radical (OH°) (Leibovitz and Siegel 1980). Following this process and addition of oxygen, a peroxy radical (ROO°) is obtained, which, by reaction with another unsaturated fatty acid, forms a lipid hydroperoxide (ROOH) as the primary oxidation product (Priestley 1986). The production of free radicals and hydroperoxides is an autocalytic autoxidation reaction because each break of a lipid double bond produces two free radicals each of which can in turn induce a break at another double-bond. The progressive inactivation of enzymes, denaturation of other proteins, and disruption of DNA and RNA slowly destroys the functioning of a cell (Harrington 1973, Bewley and Black 1985).

Lipid autoxidation is accelerated by high temperature and inhibited by the exclusion of oxygen (Schultz et al. 1962). The one anomalous factor is seed moisture. Below moisture levels at which fungi destroy viability, the drier the seed the greater the amount of lipid autoxidation. Harrington (1973) states that seed longevity increases as seed moisture is lowered from 12-14 % to 4-6 %. Below 4-6 % seed moisture, longevity decreases in line with increased lipid autoxidation. Therefore, lipid autoxidation only becomes serious below 4-6 % moisture and other factors must be more important above this level. Moreover, the lipid contents of the seed might be an important factor. Consequently, only very dry seeds that are rich in storage lipids could have been exposed to chromosome damage as a consequence of lipid peroxidation-mediated-free radical injury.

From the standpoint of the above hypothesis, it is perhaps unfortunate that evidence in favour of the free radical hypothesis is somewhat weak, and there is no direct evidence for the buildup of free radicals within aged seeds.

4. LOSS OF DNA INTEGRITY

Current thought is that loss of viability occurs due to loss of DNA integrity. The first evidence for the presence of naturally occurring breaks in

DNA with loss of viability was provided by Cheah and Osborne (1978). They showed the reduction in molecular weight of DNA with loss of viability by first isolating nuclei and then either lysing them directly on alkaline sucrose density gradients or subjecting the extracted DNA to electrophoretic separations on polyacrylamide or agarose gels. This loss of DNA integrity could be the source of chromosomal aberrations and impaired transcription observed during germination of low viability seeds. Further evidence comes from the work of Elder et al. (1987), who showed that fragmentation of nuclear DNA and loss of DNA integrity, occur progressively in the embryos of aged rye seeds. In addition, Dandoy et al. (1987) concluded that the number of apurinic or apyrimidinic (AP) sites found in DNA of radicle cells of maize embryos decreased after 2 years of storage at 20°C. Therefore, they suggested that the damage which has accumulated in DNA during storage was related to a decrease in the number of AP sites.

Recently, some biochemical and molecular biological parameters, related to damage to chromosomes as seeds age, have been investigated by several researchers. Guy et al. (1991) used restriction fragment-length polymorphism (RFLP) techniques to detect damage to DNA. They detected ageing-induced chromosome changes by the use of molecular probes in wheat seeds, and these changes were first detectable after 12 hours of ageing at 45°C. In another experiment carried out by Kraak et al. (1992), DNA was isolated from dry tomato seeds, from seeds at several stages of imbibition, from seedlings and from leaves of plants grown from control and aged seeds. To date, 7 restriction enzymes in combination with 15 probes have been examined. Probes for single copy and repetitive DNA were used. However, they have not yet succeeded in detecting changes in DNA and they have, therefore, concluded that RFLP techniques appear not suitable to detect chromosome breakage and other changes in DNA due to ageing of tomato seeds.

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